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Wound healing potential of methanolic extract of *Trichosanthes dioica* Roxb (fruits) in rats

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ABSTRACT

Aim of the study: The present study provides a scientific evaluation for the wound healing potential of methanolic (MeOH) extract of TDR fruits.
Materials and methods: Excision and incision wounds were inflicted upon three groups of six rats each. Group I was assigned as control (ointment base), Group II was treated with standard silver sulfadiazine (0.01%) cream. Group III was treated with 5% MeOH extract ointment. The parameters observed were percentage of wound contraction, epithelialization period, hydroxyproline content, tensile strength including histopathological studies.
Results: It was noted that the effect produced by the extract ointment showed significant ($P < 0.01$) healing in both the wound models when compared with control group. All parameters such as wound contraction, epithelialization period, hydroxyproline content, tensile strength and histopathological studies showed significant changes when compared to control.
Conclusion: The result shows that TDR extract ointment demonstrates wound healing potential in both excision and incision models.

1. Introduction

Herbal medicines have been enjoying revitalization among the clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very crucial and needs imperative attention in order to know the value of the plant. The assessment of the plants for their therapeutic activity is done on the basis of either their chemotaxonomic examination or ethnobotanical information for a particular disease (Juneja et al., 2007).

Trichosanthes dioica Roxb (TDR), family Cucurbitaceae (named pointed gourd) is a dioecious plant found in the plains of Northern India, extending to Assam and Bengal. Pointed gourd (TDR) is one of the most nutritive cucurbit vegetables and it holds a desirable position in the Indian market during the summer and rainy seasons. It is a perennial crop, highly accepted due to its availability for 8 months in a year (February–September). Being very rich in protein and vitamin A, it has certain medicinal properties and many reports are available regarding its role in lowering of blood sugar and serum triglycerides (Sheshadri, 1990). The fruits are 5 to 9 cm, oblong, nearly spherical acute and smooth orange red when ripe. The seeds are ellipsoid, compressed and corrugated on the margin. The plant is alternative and the fruits possess cardiotonic and anthelmentic properties (Kirtikar and Basu, 1956). The fruits are easily digestible and diuretic in nature. They are also known to have antiulcerous effects (Som et al., 1993). The leaves of the plant are cordate, rigid, sinuate-dentate and rough on both surfaces. The leaves have been claimed for glycemic property (Rai et al., 2008). The juice of the leaf is useful to patches of alopecia areata (Prajapathi et al., 2003). The seeds are reported to have anti-hyperglycemic properties. In view of these cited activities, observations and traditional uses of plant, the present study was undertaken to explore the wound healing potential of methanolic extract of TDR in excision and incision experimental models.

2. Materials and methods

2.1. Plant material

The fruits of TDR were purchased from the local market of Bhopal (India) (2008). A voucher specimen no. Bot/Herbarium/0532 has been deposited in the Department of Botany, MVM College, Bhopal (M.P.), India. The fruits were air dried in shade, powdered by a mechanical grinder, passed through a 40-mesh sieve and stored in well-closed container for future use.

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2.2. Extraction of plant drug

The powdered fruits (125 g) of *Trichosanthes dioica* were extracted with methanol using soxhlet apparatus for 24 h until the extraction was completed. The solvent was removed under reduced pressure. The dried extract was weighed and the yield was 16.35 g (w/w) with respect to dry powdered material.

2.3. Preliminary phytochemical screening

Methanolic extract of TDR was subjected to various qualitative tests for the identification of various plant constituents present in this species (Kokate, 1994; Harborne, 1998).

2.4. Preparation of formulation and standard used

A 5% (w/w) simple ointment containing the methanolic extract of TDR was prepared by trituration method in a ceramic mortar and pestle using white soft paraffin base, obtained from S.d. fine chemical, India (Cooper and Gunn's, 1987). For this, 5 g of extract was incorporated in 100 g of the base. Silver sulfadiazine (0.01%) obtained from Rexin Pharmaceutical Pvt. Ltd. was used as standard drug for comparing the wound healing potential of extract in different animal models.

2.5. Animals

Healthy albino rats of either sex (150–170 g) were selected for all the present in vivo studies. The animals were fed on normal diet and water *ad libitum*. The animals were used after an acclimatization period of 7 days to the laboratory environment. Animal study was performed in Division of Pharmacology, VNS Institute of Pharmacy, Bhopal with due permission from Institutional Animal Ethical Committee (Registration No. 778/03/c/CPCSEA).

2.6. Grouping of animals

Three groups of animals containing six in each were used for excision and incision wound models. The animals of groups I, II and III were considered as the control, reference standard and treated respectively.

2.7. In vivo studies

2.7.1. Excision wound model

The animals were divided into three groups with six in each were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 mm^2 full thickness of skin from a predetermined shaved area (Saha et al., 1997). The wounds were left undressed to the open environment. The ointment base, standard drug ointment (0.1% silver sulfadiazine) and methanolic extract of TDR ointment (5%, w/w) was applied topically to the control group, standard group and treated group respectively, till the wound was completely healed. In this model, wound contraction and epithelialization period was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination (Taranalli et al., 2004; Anderson, 1980).

2.7.2. Incision wound model

In incision wound model (Ehrlich and Hunt, 1968), all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. After the incision was made the both edges of skin kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then ointment base, standard ointment and extracts ointment were applied daily up to 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer (Hemalata et al., 2001).

3. Wound healing evaluation parameters

3.1. Measurement of wound contraction

An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated (Sadaf et al., 2006). The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm^2, as 100%, by using the following formula as

$$\%\,\text{wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{initial wound size}} \times 100$$

3.2. Epithelialization period

It was evaluated by noting the number of days required for the Escher to fall off from the wound surface exclusive of leaving a raw wound behind (Rashed et al., 2003).

3.3. Measurement of tensile strength

The force required to open the healing action is known as tensile strength. It is used to measure the completeness of healing. It also indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed on the 9th day after wounding and the tensile strength was measured on 10th day. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer, which is based on method of Kuwano et al. (1994). In this method, wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

3.4. Hydroxyproline estimation

Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60–70 °C to constant weight and were hydrolysed in 6N HCl at 130 °C for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M

Table 1
Effect of methanolic extract of *Trichosanthes dioica* and standard ointment on % of wound contraction of excision wound models in rats.

Groups	Post-wounding days										
	2	4	6	8	10	12	14	16	18	20	22
Group I	9.375 ± 0.384%	14.56 ± 0.879%	25.93 ± 0.499%	32.09 ± 1.232%	44.83 ± 0.421%	51.75 ± 0.650%	62.12 ± 1.271%	68.99 ± 0.389%	80.32 ± 0.922%	94.51 ± 0.321%	99.33 ± 0.431%
Group II	38.12 ± 1.175%*	58.13 ± 4.786%*	84.50 ± 2.49%*	93.47 ± 0.541%*	100%	–	–	–	–	–	–
Group III	18.47 ± 0.891%*	35.06 ± 0.760%*	58.07 ± 1.715%*	72.69 ± 1.201%*	80.32 ± 1.519%*	94.06 ± 0.267%*	100%	–	–	–	–

n = 6 albino rats per group; values are represents mean ± SEM.
* P < 0.01 (comparison of I with II and III).

perchloric acid and color was developed with the help of Ehrlich reagent at 60 °C. The absorbance was measured at 557 nm using a spectrophotometer (Shimadzu 1700, Pharmaspect, Japan). The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure L-hydroxyproline (Woessner, 1961).

3.5. Histopathological examinations

A specimen sample of skin tissues from control, standard and treated groups was taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin (McManus and Mowry, 1965) and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation, and angiogenesis.

3.6. Statistical analysis

Results obtained from both wound models have been expressed as mean ± SEM and the treated group was compared with control group. The results were analyzed statistically using Dunnet test followed by one-way ANOVA, to analyze the differences between the treated and control. The data were considered significant at $P < 0.01$.

4. Results

4.1. Wound contraction

A better healing pattern with complete wound closure was observed in standard and treated group within 10 and 14 days respectively while it was about 22 days in control rats as shown in Table 1.

4.2. Epithelialization period

The epithelialization time was measured from the first day. The epithelialization time was found to be significantly ($P < 0.01$) reduced in groups II and III as depicted in Table 2. The treatments with methanolic extract as well as conventional wound treatment cream were superior to control groups, which received ointment base.

4.3. Tensile strength of incision wound model

Tensile strength for the treated group on 10th day was found to be significant ($P < 0.01$) than control group as shown in Table 3.

4.4. Hydroxyproline content

Treated group showed significant increase in hydroxyproline content when compared to control group ($P < 0.01$) as depicted in Table 3.

Table 2
Effect of topical application of methanolic extract of *Trichosanthes dioica* on epithelization period.

Groups	Epithelization period (days)
Group I	21.25 ± 0.4787
Group II	9.5 ± 0.2887*
Group III	11.5 ± 0.6445*

n = 6 albino rats per group; values represents mean ± SEM.
* P < 0.01 (comparison of I with II and III).

Table 3
Effect of methanolic extract of *Trichosanthes dioica* and standard ointment on various wound parameters of incision wound model in rats.

Groups	Hydroxyproline (mg/g tissue)	Tensile strength (g/mm^2)
Group I	21.33 ± 0.001	415.03 ± 4.167
Group II	72.66 ± 0.004*	605.22 ± 3.717*
Group III	61.33 ± 0.003*	569.80 ± 3.665*

$n = 6$ albino rats per group; values represents mean ± SEM.
* $P < 0.01$ (comparison of I with II and III).

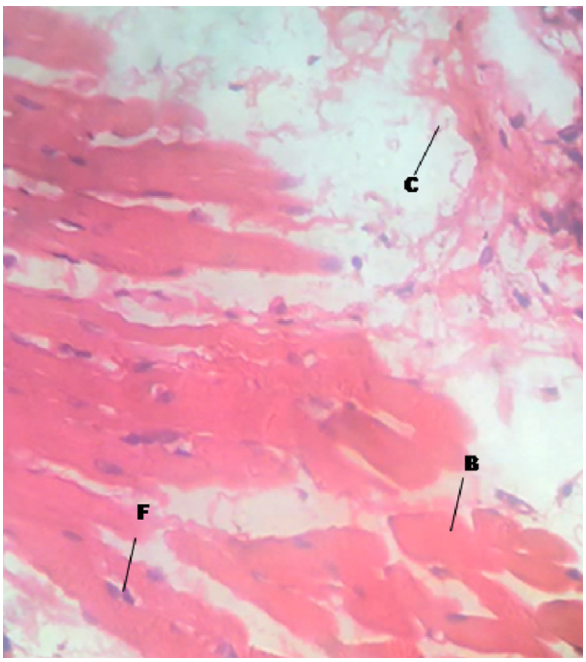

Fig. 1. Hepatological characteristics of healed tissue on 8th day by treatment with standard ointment. Figure shows increased fibroblasts cells (F), collagen fibres (C) and blood vesicles (B) in incision wound.

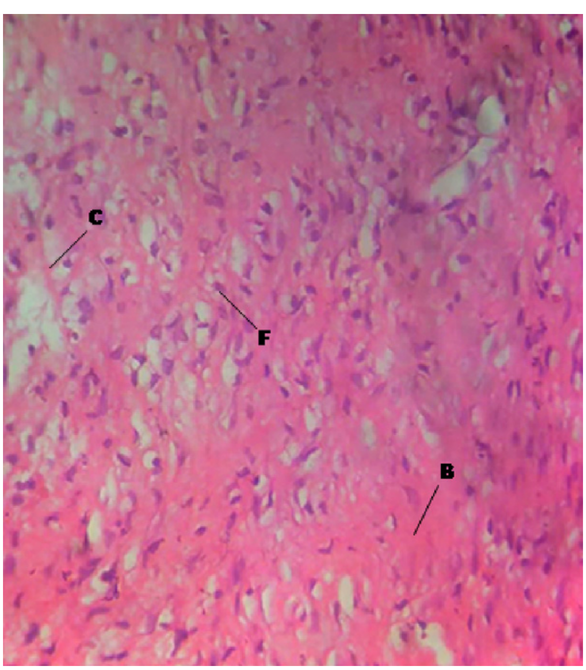

Fig. 2. Histopathological characteristics of rat skin on 8th day by treatment with 5% methanolic ointment. Figure shows increased fibroblast cells (F), blood vessels (B) and collagen fibres (C) in incision wound.

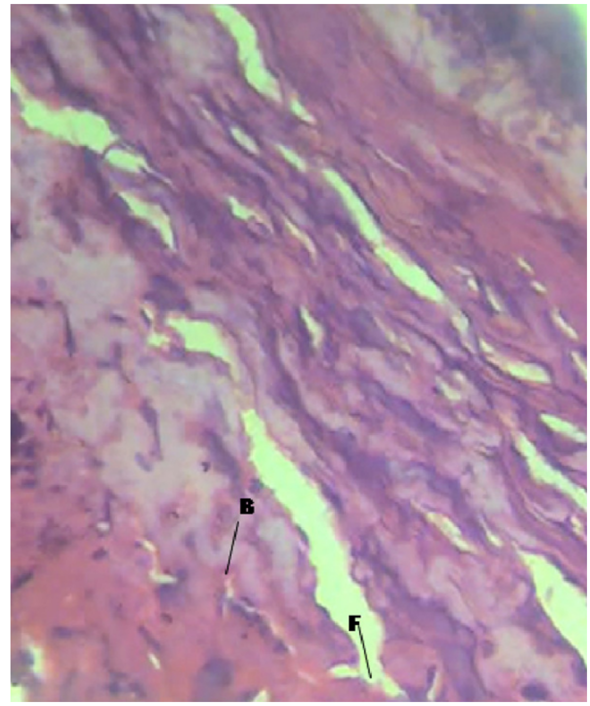

Fig. 3. Histopathological characteristics of rat skin on 8th day treatment with ointment base. Figure shows poor fibroblast cells (F) and blood vessels (B) in incision wound.

4.5. Histopathological examinations

In standard and treated albino rats, excision and incision type of wounds have shown significant healing as in fibroblasts cells (F), collagen fibres (CF) and new blood vesicles (BV) in Figs. 1 and 2 while in control rats wounds shown incomplete healing in Fig. 3. Control group has shown to slightest wound healing ability when compared to extract treated and reference ointment group. Fibroblast cells, collagen fibres and blood vessels are prominently present in standard and extract treated group as compared to control.

5. Discussions and conclusion

Wound healing is stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair (Charles et al., 1995). Hence in this study, excision and incision wound models were used to evaluate the effect of methanolic extract ointment on various phases.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres (Udupa et al., 1995). Increase in blood vessels and role of antioxidants were experimentally proved (Michel and Fredrickson, 1990). In excision wound, the methanolic extract showed faster healing with earlier wound contraction compared with control groups. The earlier wound contraction rate of the methanolic extract may be due to stimulation of interleukin-8, an inflammatory a-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue (Moyer et al., 2002). The methanolic extract of TDR increased cellular proliferation and collagen synthesis at the wound site as evidenced by increase in total protein and total

collagen contents reflected by hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extra cellular matrix of skin, joints, eyes and many other tissues and organs. In spite of its simple structure, it demonstrates remarkable visco-elastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin are due to its interaction with various binding proteins. Due to an influence on signaling pathways, hyaluronic acid and hydroxyproline is involved in the wound-healing process and scarless fetal healing. In clinical trials, topical application of hyaluronic acid has improved the healing of wound (Weindl et al., 2004). In addition, the muco-polysaccharide hyaluronic acid protects granulation tissue from oxygen free radical damage and thereby stimulates wound healing (Bayliss, 1984). Among the glycosaminoglycans, hydroxyproline, dermatan sulfate and dermatan have also been implicated in wound repair and fibrosis. Their ability to bind and alter protein–protein interactions has identified them as important determinants of cellular responsiveness in development, homeostasis and disease (Trowbridge and Gallo, 2002). In our study, hydroxyproline content was significantly increased ($P < 0.01$) when compared with control. Since TDR extract has increased the levels of these compounds considerably, it is likely that the observed increase in tensile strength was not only due to increased collagen synthesis but also due to its proper deposition and alignment. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Overproduction of reactive oxygen species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore, elimination of ROS could be an important strategy in healing of chronic wounds (Dissemond et al., 2002). Therefore, estimation of antioxidants like SOD, catalase and glutathione in granulation tissues is also relevant because these antioxidants hasten the process of wound healing by destroying the free radicals (Halliwell et al., 1988). The significant alteration in the antioxidant profile accompanied by the elevated levels of MDA, a marker of free radical damage, may be attributed to impaired wound healing in immunocompromised rats (Gupta et al., 2002). While numerous attempts have been made to identify prognostic biomarkers of wound healing in skin, these have met with limited success.

The results showed that methanolic extract ointment possesses a distinct prohealing stroke. This was demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelialization period. Significant increase ($P < 0.01$) in tensile strength, and hydroxyproline content were observed, which was auxiliary supported by histopathological studies. This indicated newly formed fibroblasts cells, collagen fibres and blood vessels. Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids (Tsuchiya et al., 1996), triterpenoids (Scortichini et al., 1991) and tannins (Rane et al., 2003) are known to promote the wound-healing process.

Preliminary phytochemical screening of methanolic extract of TDR showed the presence of alkaloids, flavonoids and tannins. The chemical constituents of Trichosanthes plants (*Trichosanthes kirilowii*, *Trichosanthes rosthornii*, *Trichosanthes anguina*, *Trichosanthes cucumeroides*, *Trichosanthes tricuspidata*, *Trichosanthes dioica*, *Trichosanthes integrifolia*, *Trichosanthes damiaoshanensis*, and *Trichosanthes truncata*) and their medicinal values have been reviewed. Its chemical constituents mainly consist of oils and fats, org. acids, flavonoids, triterpenes, steroids, sterols, and proteins (Chen et al., 2006). The wound healing action of TDR may probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents.

Hence, the results obtained from data concludes that methanolic extract ointment of TDR has properties that render it capable of promoting wound healing activities such as stimulating wound contraction and increasing tensile strength of incision as compared to control.

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